#7 Election

PATENT
Docket No.: **21829/230 (EBC-015)**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Zhong-Min Wei	Examiner: A. Kubelik
Serial No.	:	10/034,158	
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For	:	METHOD OF IMPARTING DROUGHT RESISTANCE TO PLANTS	

RESPONSE TO RESTRICTION REQUIREMENT

U.S. Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

In response to the January 21, 2003, written restriction requirement, applicants hereby elect, with traverse, hypersensitive response elicitor proteins or polypeptides of *Erwinia amylovora*.

Applicants hereby traverse the restriction between the various hypersensitive response elicitor proteins or polypeptides on several grounds.

Firstly, the U.S. Patent & Trademark Office ("PTO") has improperly required restriction among the various hypersensitive response elicitor proteins. The asserted basis for making this restriction is that the amino acid sequences of the various hypersensitive response elicitor proteins constitute independent and distinct inventions (see office action at pages 2-3). While the proteins themselves possess different amino acid sequences, the PTO's position ignores the relatedness of hypersensitive response elicitor proteins.

As demonstrated in the accompanying Declaration of Zhong-Min Wei Under 37 C.F.R. § 1.132 ("Wei Declaration"), hypersensitive response elicitors are an art-recognized class of proteins and, therefore, results achieved with one such protein would be expected when other proteins in this class of proteins are used. The Wei Declaration also

provides experimental data confirming that disease resistance in plants (among other results) can be achieved using hypersensitive response elicitors other than the *E. amylovora* harpin$_{Ea}$ (SEQ ID NO: 3).

In plants, the hypersensitive response phenomenon results from an incompatible interaction between plant pathogens and non-host plants (Wei Declaration ¶ 5). As explained in Gopalan et al., "Bacterial Genes Involved in the Elicitation of Hypersensitive Response and Pathogenesis," Plant Disease 80: 604-10 (1996) ("Gopalan") (attached to Wei Declaration as Exhibit 1), these types of interactions involve, for example, a bacterial plant pathogen attempting to infect a host plant, and the host plant preventing proliferation of the pathogen by the collapse and death, or necrosis, of plant leaf cells at the site of infection (Id.). This is distinct from a compatible interaction between a bacterial plant pathogen and a host plant in which the bacteria is capable of proliferation, resulting in the spread of the pathogen throughout the plant and the manifestation of disease symptoms. Gopalan at 604 (Id.).

Hypersensitive response elicitors within a given genus are often homologous to elicitors from different pathogenic species and strains of the same genus (Wei Declaration ¶ 6). For example, homologs of hypersensitive response elicitors from *Erwinia amylovora* and *Pseudomonas syringae* have been identified in different bacteria species and strains from the genera *Erwinia* and *Pseudomonas*, respectively. See Gopalan. (Id.)

In addition, numerous reported studies confirm that a gene encoding a hypersensitive response elicitor from a particular source genus can be used to isolate a corresponding hypersensitive response elicitor gene from different species and strains of that same genus (Wei Declaration ¶ 7). For example, in Bauer et al., "*Erwinia chrysanthemi* Harpin$_{Ech}$: An Elicitor of the Hypersensitive Response that Contributes to Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995) ("Bauer") (attached to Wei Declaration as Exhibit 2), the *Erwinia amylovora* hypersensitive response elicitor encoding gene was used as a probe to isolate, clone, and sequence the gene encoding the *Erwinia chrysanthemi* hypersensitive response elicitor, as follows:

> The cosmids were probed in colony blots with a 1.3-kb *Hind*III fragment from pCPP1084, which contains the *E. amylovora hrpN* gene (Wei et al. [, "Harpin Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (]1992[)]). pCPP2157, one of the three cosmids hybridizing with the probe, was digested with several restriction enzymes, and the location of the *hrpN$_{Ech}$* gene in those fragments was determined by probing a Southern blot with *E. amylovora Hind*III fragment. Two fragments, each

> containing the entire *$hrpN_{Ech}$* gene, were subcloned into different vectors: pCPP2142 contained an 8.3-kb *Sal*I fragment in pUC119 (Vieira and Messing [,"Production of Single-Stranded Plasmid DNA," Methods Enzymol., 153:3-11(] 1987[)]), and pCPP2141 contained a 3.1-kb *Pst*I fragment in pBluescript II SK(-) (Stratagene, La Jolla, CA).
>
> *Sequence of $hrpN_{Ech}$*
>
> The nucleotide sequence of a 2.4-kb region of pCPP2141 encompassing *$hrpN_{Ech}$* was determined. The portion of that sequence extending from the putative ribosome-binding site through the *$hrpN_{Ech}$* coding sequence to a putative rho-independent terminator is presented in Figure 1.

See page 485 (Id.).

In the same manner as described in Bauer *supra*, Cui et al., "The $RsmA^-$ Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp*N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996) ("Cui") (attached to Wei Declaration as Exhibit 3) demonstrates that the gene encoding the *Erwinia carotovora* hypersensitive response elicitor can be isolated, sequenced, and cloned by using the *Erwinia chrysanthemi* hypersensitive response elicitor encoding gene to probe the genomic library of *Erwinia carotovora* (Wei Declaration ¶ 8). Further, Cui (at page 572) states the following:

> The genomic library of *E. carotovora* subsp. *carotovora* strain Ecc71 in pLARF5 was screened by in situ colony hybridization with a 0.75-kb internal *Cla*I fragment of *hrpN* of *E. chrysanthemi* (Bauer et al.[, "*Erwinia chrysanthemi* $Harpin_{Ech}$: An Elicitor of the Hypersensitive Response that Contributes to Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (]1995). Two cosmids, pAKC921 and pAKC922, that hybridized with the probe were isolated. The subclones (pAKC923 and pAKC924, Table 1) carrying *hrpN* DNA were used for sequence analysis.

(Id.)

The gene encoding the hypersensitive response elicitor of *Erwinia amylovora* has also been used as a probe to isolate and clone the gene encoding the hypersensitive response elicitor of *Erwinia stewartii* (Wei Declaration ¶ 9). It was found that antibodies raised against the hypersensitive response elicitor of *Erwinia stewartii* cross-reacted with the hypersensitive response elicitor of *Erwinia amylovora*. See Ahmad et al., "Harpin Is Not Necessary for the Pathogenicity of Maize," 8th Int'l Cong. Molec. Plant Microbe Inter. July 14-19, 1996 ("Ahmad") (attached to Wei Declaration as Exhibit 4) (Id.).

Similar findings were reported for hypersensitive response elicitors from the genus *Pseudomonas* (Wei Declaration ¶ 10). An internal fragment of the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae* (i.e., *hrpZ*) was used to identify and isolate the hypersensitive response elicitors from *P. syringae* pv. *glycinea* and *P. syringae* pv. *tomato* (Id.). Significant amino acid sequence similarities were identified between the various *Pseudomonas syringae* elicitors. See Preston et al., "The HrpZ Proteins of *Pseudomonas syringae* pvs. *syringae, glycinea,* and *tomato* Are Encoded by an Operon Containing *Yersinia ysc* Homologs and Elicit the Hypersensitive Response in Tomato But Not Soybean," MPMI 8(5): 717-32 (1995) ("Preston") (attached to Wei Declaration as Exhibit 5) (Id.).

The genes encoding hypersensitive response elicitors are positioned within the *hrp* gene cluster or proximate to the *hrp* gene cluster in *hrp* regulons (Wei Declaration ¶ 11). For example, *hrpN* from *Erwinia amylovora* was located within the *hrp* gene cluster, as was *hrpZ* from *Pseudomonas syringae* (Id.). The *popA* gene, encoding a hypersensitive response elicitor from *Pseudomonas solanacearum*, was located on the left flank of the *hrp* gene cluster within a *hrp* regulon. See Bonas, "*hrp* Genes of Phytopathogneic Bacteria," Current Topics in Microbiology and Immunology 192: 79-98 (1994) ("Bonas I") (attached to Wei Declaration as Exhibit 6) and Alfano et al., "The Type III (Hrp) Secretion Pathway of Plant Pathogenic Bacteria: Trafficking Harpins, Avr Proteins, and Death," Journal of Bacteriology 179: 5655-5662 (1997) ("Alfano") (attached to Wei Declaration as Exhibit 7) (Id.). Similar to the *popA* gene, *hreX*, the gene encoding the hypersensitive response elicitor from *Xanthomonas campestris*, was located on the left flank of the *hrp* gene cluster. See Swanson et al., "Isolation of the *hreX* Gene Encoding the HR Elicitor Harpin (Xcp) from *Xanthomonas Campestris* pv. *pelargonii*," Phytopathology 90: s75 (1999) ("Swanson") (attached to Wei Declaration as Exhibit 8) (Id.).

The characteristics that distinguish hypersensitive response elicitors as a distinct class of molecules are clearly apparent when considering the different elicitors' secretion mechanisms, regulation, biochemical characteristics, and biological activities (Wei Declaration ¶ 12).

Substantially all hypersensitive response elicitors identified have been shown to be secreted through the type III, *hrp* dependent secretion pathway (Wei Declaration ¶ 13). The type III secretion pathway is a highly conserved and unique mechanism for the delivery of pathogenicity related molecules in gram-negative bacteria (Id.). The *hrp* gene cluster is

largely composed of components of the type III secretion system. See Bogdanove et al., "Unified Nomenclature for Broadly Conserved *hrp* Genes of Phytopathogenic Bacteria," Molec. Microbiol. 20:681-83 (1996) ("Bogdanove") (attached to Wei Declaration as Exhibit 9); and Alfano (Id.).

Regulation of the genes encoding the *hrp* gene cluster, and subsequently the genes encoding the components of the type III secretion system and hypersensitive response elicitors, is controlled by environmental factors (Wei Declaration ¶ 14). Specifically, transcriptional expression of these genes is induced under conditions that mimic the plant apoplast, such as low concentrations of carbon and nitrogen, low temperature, and low pH. See Wei et al., "Regulation of hrp Genes and Type III Protein Secretion in *Erwinia amylovora* by HrpX/HrpY, a Novel Two-Component System, and HrpS," MPMI 13(11): 1251-1262 (2000) ("Wei I") (attached to Wei Declaration as Exhibit 10); and Bonas I (Id.).

Biochemically, hypersensitive response elicitors have a number of common characteristics (Wei Declaration ¶ 15). These include being glycine rich, heat stable, hydrophilic, lacking of an N-terminal signal sequence, and susceptible to proteolysis. See Bonas, "Bacterial Home Goal by Harpins," Trends Microbiol 2: 1-2 (1994) ("Bonas II") (attached to Wei Declaration as Exhibit 11); Bonas I; Gopalan; and Alfano (Id.).

In addition, hypersensitive response elicitors share a unique secondary structure that has been associated with these elicitors' distinct biological activities (described below) (Wei Declaration ¶ 16). The structure has two primary components, an alpha helix unit and a relaxed acidic unit having a sheet or random turn structure (Id.). In the absence of one or both of these components, hypersensitive response elicitation does not occur. See WO 01/98501 to Fan et al. ("Fan") (attached to Wei Declaration as Exhibit 12) (Id.).

In addition to eliciting the hypersensitive response in a broad range of plant species, as explained by Wei et al., "Harpin from *Erwinia amylovora* Induced Plant Resistance," Acta Horticulture 411: 223-225 (1996) ("Wei II") (attached to Wei Declaration as Exhibit 13) and by Alfano, hypersensitive response elicitors also share the ability to induce specific plant responses (Wei Declaration ¶ 17). The induction of plant disease resistance, plant growth enhancement, and plant stress resistance are three plant responses that result from treatment of plants or plant seeds with a hypersensitive response elicitor from a gram-negative plant pathogen (Id.).

As described in Wei II, treatment of plants with the hypersensitive response elicitor HrpN from *Erwinia amylovora* resulted in disease resistance to a broad range of plant

pathogens (Wei Declaration ¶ 18). For example, HrpN induced disease resistance to southern bacterial wilt (*Pseudomonas solanacearum*) in tomato, tobacco mosaic virus in tobacco, and bacterial leaf spot (*Gliocladium cucurbitae*) in cucumber (Id.).

The hypersensitive response elicitor HrpZ from *Pseudomonas syringae* was reported to induce disease resistance in cucumber to a diverse range of pathogens, including the fungal disease *Colletotrichum lagenarium*, tobacco necrosis virus, and bacterial angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*). See Strobel et al., "Induction of Systemic Acquired Resistance in Cucumber by *Pseudomonas syringae* pv. *syringae* 61 $HrpZ_{Pss}$ Protein," Plant Journal 9(4): 431-439 (1996) ("Strobel") (attached to Wei Declaration as Exhibit 14) (Wei Declaration ¶ 19).

The Wei Declaration demonstrates that the hypersensitive response elicitor from *Xanthomonas campestris* pv. *pelargonii* (i.e., HreX) induced disease resistance in tomato against bacterial wilt caused by *Pseudomonas solanacearum*, as well as disease resistance in tobacco against tobacco mosaic virus (Wei Declaration ¶¶ 21-23).

Thus, application of a broad range of hypersensitive response elicitors to plants have been shown to induce disease resistance.

Hypersensitive response elicitors from *Erwinia amylovora* and *Pseudomonas syringae* pv. *syringae* are known to enhance plant growth. See Examples 1 to 24 of U.S. Patent No. 6,277,814 to Qiu et al. ("Qiu") (attached to Wei Declaration as Exhibit 15), which showed that treatment of plants and plant seeds with HrpN from *E. amylovora* induced plant growth enhancement in species of tomato, potato, raspberry, and cucumber (Wei Declaration ¶ 20). This is further demonstrated by the Wei Declaration, which shows that treatment of tomato seeds with the hypersensitive response elicitors from *Pseudomonas syringae* pv. *syringae* (i.e., HrpZ) and *Xanthomonas campestris* pv. *pelargonii* (i.e., HreX) independently resulted in significant increases in plant height over that of the buffer treated control (Wei Declaration ¶¶ 24-26). Thus, application of a broad range of hypersensitive response elicitors has been shown to enhance plant growth.

Hypersensitive response elicitors from *Erwinia amylovora* are known to impart resistance to various forms of plant stress resistance. As evidenced by the experimental results reported in Example 12 of the above-identified application and from Examples 1-6 of WO 00/28055 to Wei et al. (attached to Wei Declaration as Exhibit 16), HrpN from *Erwinia amylovora* is capable of inducing various forms of plant stress resistance, such as chemical stress resistance, drought stress resistance, and nutritional stress resistance

(Wei Declaration ¶ 27). In addition, the Wei Declaration shows that treatment of plants with HreX from *X. campestris* pv. *pelargonii* induces various forms of plant stress resistance. For example, the treatment of corn with HreX was shown to induce chemical stress resistance and the treatment of lima beans with HreX was shown to induce salt stress resistance (Wei Declaration ¶¶ 28-32). Thus, application of a broad range of hypersensitive response elicitors has been shown to impart stress resistance to plants.

From all of the foregoing literature and cited data, there is ample support to show that hypersensitive response elicitors from plant pathogens are an art-recognized class of proteins which achieve a variety of common beneficial effects in plants. Therefore, restriction among the various hypersensitive response elicitors is improper and should be withdrawn.

Secondly, from a practical perspective, although the amino acid sequences for the above-identified hypersensitive response elicitors were known previously, applicants included them in the application in part to satisfy the written description requirement under 35 U.S.C. § 112 (first paragraph) for the claimed genus. In particular, each of the proteins of SEQ ID NOs: 1, 3, 5, and 7 was known in the art, having been independently patented or otherwise disclosed in the literature as follows:

SEQ ID NO: 1 – e.g., U.S. Patent No. 6,001,959 to Bauer et al.;
SEQ ID NO: 3 – e.g., U.S. Patent No. 5,849,868 to Beer et al.;
SEQ ID NO: 5 – e.g., U.S. Patent No. 5,708,139 to Collmer et al.; and
SEQ ID NO: 7 – e.g., Arlat et al., EMBO J. 13:543-533 (1994).

In the present application, applicants are merely claiming the use of such proteins generically within the limitations of claims 1-9. Imposing a restriction requirement for purposes of now limiting applicant's claimed invention to the use of hypersensitive response elicitor proteins from a single bacterial species (*Erwinia amylovora*, as elected) negates the breadth of the invention as claimed and effectively defeats the purpose for which applicants included such matter in the first place.

For all these reasons, applicants submit that the restriction among the various hypersensitive response elicitors is improper and, therefore, the restriction requirement should be withdrawn in its entirety.

Respectfully submitted,

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